Prepulse Inhibition Modulation by Contextual Conditioning of Dopaminergic Activity

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Abstract

When a neutral stimulus is repeatedly paired with a drug, an association is established between them that can induce two different responses: either an opponent response that counteracts the effect of the drug, or a response that is similar to that induced by the drug. In this paper, we focus on the analysis of the associations that can be established between the contextual cues and the administration of dopamine agonists or antagonists. Our hypothesis suggests that repeated administration of drugs that modulate dopaminergic activity in the presence of a specific context leads to the establishment of an association that subsequently results in a conditioned response to the context that is similar to that induced by the drug. To test this hypothesis, we conducted two experiments that revealed that contextual cues acquired the property to modulate pre-pulse inhibition by prior pairings of such context with the dopamine antagonist haloperidol (Experiment 1), and with the dopamine agonist d-amphetamine (Experiment 2). The implications of these results are discussed both at a theoretical level, and attending to the possibilities that could involve the use of context cues for the therapeutic administration of dopaminergic drugs.

Key words: Dopamine; Context Conditioning; Prepulse Inhibition

1. Introduction

Classical conditioning has been considered for more than a century as one of the more flexible ways of learning used by those organisms with a complex nervous system in order to adapt to the demands of a continuously changing environment [1]. The implications of this type of learning have gone beyond the study of the processes by which the associations between stimuli are established, reaching areas such as the study of eating habits [2], emotional processes [3], or the analysis and treatment of some pathological behaviors [4], to mention only some of the most relevant applied areas in this field of research.

One additional potential area of interest related to Pavlovian learning is the analysis of the associations that can be established between a neutral stimulus and the effects of certain drugs [5]. In this domain, two main fields of research can be identified: one that has led to results that show what has been called an "opponent process", by which the Conditioned Stimulus (CS) induces a response that is the opposite to that produced by the drug [6,7,8]. Conversely, a second set of results indicate that the association between a neutral stimulus and the drug results in a Conditioned Response (CR) similar to that produced by the drug [9,10].

In an attempt to make compatible both sets of results, Eikelboom and Stewart [5] proposed that the functioning of the drug-response regulation system is based on a comparison between whether the drug has any direct effect or not in the central nervous system. From this perspective, a hypothetical response generator (or “integrator”) receives the inputs from the afferent or efferent arms of the feedback systems. Those physiological changes induced by the drug that access the response generator from an afferent neural path act

as a signal to activate an effector that will be responsible for the observed drug effect. In such cases, the drug effect corresponds to an Unconditional Response (UR) that can be associated with a neutral stimulus to induce a CR that is similar to that induced by the drug. On the other hand, other drugs act on the efferent arm of the feedback system (affecting, for instance, the effector organ directly). The effect of such drugs cannot be considered as an UR, but the physiological changes induced by the drug may result in a signal to the response generator that will activate the efferent path to counteract the disturbance produced by the drug. Such an opposite response can in fact be considered from this perspective as an UR that could be associated with a neutral stimulus through an associative process. An example of this situation is, for instance, the hypothermia caused by ethanol [6,11]. Following Eikelboom & Stewart's proposal [5], ethanol directly acts on the efferent arm of the thermoregulatory system causing a drop in body temperature that acts as a signal for the response generator to activate an increase in body temperature through the effector's activation. As a result of this process, a neutral stimulus associated with ethanol will result in a CR manifested as an increase in body temperature (a response that is opposite to the direct action of the drug).

On the other hand, there have also been numerous studies of conditioning in which the stimuli associated with a drug elicited a CR that is similar to the direct effect of the drug. This area of research finds its most remote precedents in the work by Pavlov and his associates, who described a report in which, after several morphine injections, the mere presence of the context in which the drug was administered induced the same increase in the salivary response that was observed after morphine administration [12]. This

study was pioneering in demonstrating that pairing the contextual cues with the drug administration can result in a conditioning process by which the context acts as a CS that mimics the effect of the drug. Subsequent studies using cocaine demonstrated that an association of the drug administration with a specific context resulted in an increase of the effects produced by the drug in the presence of the conditioned context [13,14]. Ross and Schnitzer [15] observed similar effects when injecting amphetamine into rats and, since then, there have been numerous experimental demonstrations of an augmentation in locomotor activity in the presence of a CS previously associated with amphetamine administration [16,17,18,19]. Considering the above-described proposal by Eikelboom and Stewart [5] it can be hypothesized that amphetamine administration is generating an increase of dopaminergic activity that would be the basis of the increase in the locomotor activity [20,21] that would be detected by the regulatory system through the afferent arm. Therefore, the action of amphetamine serves as an Unconditioned Stimulus (US) to increase dopamine activity (the UR), and the expected CR is in the same direction as the observed effect of amphetamine.

In the following experiments we evaluated Prepulse Inhibition (PPI) in the presence of a context previously associated with the administration of a dopamine agonist (d-amphetamine) or antagonist (haloperidol). PPI is considered an example of sensory-motor gating, a process that impedes the processing of a stimulus in order to protect the processing of the stimulus that is already in progress [22], and can be easily reproduced in experimental conditions by presenting a low-intensity stimulus (typically a tone), named "Prepulse", for a short time (typically 80 -120 ms) preceding a stronger stimulus

(called "Pulse"). The result is a reduction of the startle response to the pulse when it is preceded by the prepulse as compared to the pulse alone [23,24]. PPI occurrence and intensity depend on several variables like the intensity of the stimuli presented [25,26], the length of the temporal interval between the prepulse and the pulse [25,27], or the intensity of the background noise [28,29].

The physiological basis of PPI has been described in detail and, in particular, it seems to be modulated by several neurotransmitters including dopamine, GABA, glutamate, and acetylcholine, which regulate the magnitude of the startle response and its inhibition [23]. There is evidence demonstrating that dopamine-agonist administration results in a significant reduction of the PPI effect [30,31,32]. In addition, such dopamine-mediated PPI reduction is counteracted by the administration of haloperidol, a D2 and D3 dopamine antagonist [30], and the administration of haloperidol by itself has been shown to increase PPI [33].

Attending to the described results regarding drug conditioning, and PPI modulation by dopamine agonists, we propose that evaluating PPI in the presence of a context that had been repeatedly paired with a dopamine agonist or antagonist will result in a CR induced by the context that will modulate PPI intensity in the same way that the drug would do (namely, a PPI reduction with dopamine agonist and a reversion of the effect with dopamine antagonist). In order to test this hypothesis, we ran two experiments in which we associated a specific set of contextual cues with repeated administration of a dopamine agonist (d-amphetamine) or antagonist (haloperidol). In Experiment 1, one group received three pairings of a specific context and amphetamine injections, and a second group received the same number of pairings of the context with

the administration of haloperidol prior to the amphetamine injection. In the test stage, all animals in both groups received a dose of amphetamine and PPI intensity was registered in the presence of the context previously associated with the corresponding drugs in order to be compared with PPI that had been registered before the start of the drug administration. In Experiment 2, for one group the context was associated with amphetamine administration and for a second group with haloperidol injections. During testing, PPI was evaluated in the presence of the corresponding conditioned context (in this case the animals were injected with a saline solution) in order to compare PPI intensity with that registered before the start of the experimental treatments. We used a PPI protocol previously validated in the absence of any drug treatment [34]. We added additional trials with 80 Db and 100 Db to the mentioned protocol in order to get a more ample range of PPI intensities.

2. Experiment 1

In this experiment, a group of rats received alternated i.p. amphetamine injections in the presence of a specific set of contextual cues, and haloperidol followed by d-amphetamine i.p. injections in the presence of a second different context. In the test stage, all animals were injected with amphetamine and PPI was evaluated both in the presence of the context associated with amphetamine and in the context associated with haloperidol + amphetamine. Context conditioning of dopaminergic activity would be demonstrated if PPI tested in the context associated with the dopamine agonist is lower as compared to PPI tested in the context paired with the dopamine antagonist.

2.1 Method.

2.1.1.Subjects.

16 male Wistar rats (n=8) experimentally naïve, participated in this experiment. Mean weight at the start of the experiment was 336 g. (range 317-394). Food and water were available ad libitum throughout the experiment. Rats were individually housed in the colony room with a regular light-dark cycle of 12:12 hours. Four days before the start of the experimental sessions, each of the animals was handled 5 min daily. All procedures were conducted in accordance with the guidelines established by Directive 86/609/CEE of the European Community Council, and the Spanish R.D. 223/1988.

2.1.2. Apparatus

Four Panlab chambers (model LE 111) designed to detect and record the startle response in rats were used. Each chamber was enclosed in a soundproofed module (model LE 116), and inside each chamber a Plexiglas cylinder 8 cm in diameter was attached to the floor of the experimental chamber, resting on a platform that registered and recorded each animal´s movement. Vibrations of the Plexiglas enclosure caused by the whole-body startle response of the animal were converted into analog signals by a piezoelectric unit attached to the platform. These signals were digitized and stored by a computer as a linear parameter. The average startle activity was measured in a 100-ms time window starting at the onset of the sound stimulus.

At the top of the camera there was a loudspeaker, which produced a constant background white noise of 65 dB. The pulse was a 20 ms, 120 dB white noise, and the pre-pulses were 20 ms, 80, 90, and 100 dB white noise. The lead interval for the prepulse-pulse trials was 100 ms, and intertrial interval between Pulse-alone and Prepulse-Pulse presentations was 30 sec (+/- 5). In

order to get dissimilar contexts two different odors were used on each one (mint vs. almond). Additionally, for context A a 24V 2W keylight located in the left side of the chamber was switched on for the entire duration of each session, while it was switched off for context B. Amphetamine (3 mg/kg.) was dissolved in saline solution. Haloperidol (2 mg/Kg) was first dissolved in a few drops of acetic acid and then in 100 ml of saline (final pH: 6).

2.1.3. Procedure

For every animal, PPI was registered using the following protocol: Once the rats were introduced in the experimental chamber went through a 5-minute acclimation period in which the only auditory stimulation presented was the constant 65-dB SPL background noise, which remained throughout the experiment. After the acclimation period, 5 pulses were delivered in order to stabilize the startle response, with a mean ITI of 30 s. (+/-5). After 30 additional sec, 15 pulse-alone and 15 prepulse-pulse trials (5 with the 80 Db Prepulse, 5 with the 90 Db Prepulse, and 5 with the 100 Db Prepulse) were presented in five blocks that included random presentation of 3 pulse-alone and three trials with each prepulse-pulse value. The ITI was 30 sec (+/- 5 sec).

The effect of prepulses on the startle response was determined as the difference between responses in pulse-alone and prepulse-pulse trials, and expressed as percent PPI: PPI% = 100 x ([RP – RpP] / RP) where RP represents the average startle amplitude in Pulse-alone trials, and RpP indicates the average startle amplitude in prepulse-pulse trials, that was independently calculated for each prepulse value (80, 90, and 100 dB). The use of mean average startle amplitude collapsed across Pulse-alone trials in the formula was intended to avoid a possible effect of the higher number of Pulse-

alone trials (a total of 15) as compared to number of trials for each prepulse condition (5 for each intensity value).

A summary of the experimental procedure is provided in the upper section of Table 1. In order to obtain a baseline of mean PPI in absence of drugs, PPI was registered for half of the animals in presence of Context A (mint-light), and for the other half in presence of Context B (almond-dark). Thirty min before the start of each baseline PPI session the animals received an i.p. injection of saline (1 ml /Kg).

Table 1 about here

Context conditioning sessions were conducted in three blocks of 4 days each. In order to counterbalance contexts, for days 1 and 2 on each block half of the animals on each group received amphetamine in context A and Haloperidol + Amphetamine in context B. For the other half, the contexts were reversed on days 3 and 4. Therefore, on each 4-day block every animal received one association between the correspondent context and amphetamine and one pairing between the alternative context and haloperidol + amphetamine. In order to simplify the description of the procedure we will not make any additional reference to context counterbalancing.

For the context-amphetamine trials each animal was i.p. injected with the drug and introduced in the experimental context and remained undisturbed for 30 min. Next, the PPI protocol was initiated. For the haloperidol + amphetamine trials, haloperidol was i.p. injected, the animals were exposed to the corresponding context for 20 min, then they were injected with amphetamine,

introduced in the context for an additional 30 min period, and finally exposed to the PPI protocol.

Test stage was conducted on days 15 and 16. The first test day all rats received an amphetamine injection and were introduced in Context A (that had been associated with amphetamine for half of the animals and with amphetamine + haloperidol for the other half). After 30 min. without any additional manipulation the PPI protocol was initiated. The second test day was exactly as described except that it was conducted in Context B.

2.2. Results

A preliminary 3 x 2 mixed ANOVA (Prepulse Intensity: 80 vs. 90 vs. 100 x Context: A vs. B, the first factor within-subject) was conducted on mean percent PPI during the first two days of the experiment (baseline). Only the main effect of Prepulse Intensity was significant, $F(2,28)=4.27$; $p<.05$ (all remaining ps>.23), revealing that the context differences did not differentially affect PPI. T-tests for related samples ($p<.05$, two-tailed) revealed that the main effect of prepulse intensity was due to a higher percent PPI in the 90 dB condition (mean = 56.43%, SD = 16.58) as compared to the 80 dB and 100 dB conditions (mean = 44.38%, SD = 20.13, and mean = 39.31%, SD = 21.42, respectively).

To evaluate the effect of the drug administration on PPI intensity a 3 x 2 ANOVA (Prepulse Intensity: 80 vs 90 vs 100 Db x Drug: Amphetamine vs. Haloperidol + amphetamine, both factors within-subject) was conducted on mean PPI collapsed across context conditioning sessions. The analysis revealed significant main effects of Prepulse Intensity and Drug main factors,

F(2,30)=33.00; p<.001, and F(1,15)=33.58; p<.001, respectively. The 2-way interaction was non-significant, F(2,30)<1. T-tests for related samples (p<.05, two-tailed) revealed that the main effect of Prepulse Intensity was due to a higher overall percent PPI in the 90 dB (mean = 54.14%, SD = 11.21) as compared to the 80 dB and 100 dB conditions (mean = 36.84%, SD = 13.18, and mean = 28.18%, SD = 11.17, respectively). The main effect of Drug reflects the general reduction in percent PPI for those trials with amphetamine administration (mean = 26.48%, SD = 15.45) as compared to the haloperidol + amphetamine trials (mean = 45.98%, SD = 14.25, respectively).

In order to evaluate the effect of context conditioning, a 3 x 3 ANOVA (Context test: Baseline vs. Amphetamine vs. Haloperidol + Amphetamine x Prepulse Intensity: 80 dB vs. 90 dB vs. 100 dB, both factors within-subject) was conducted on mean percent PPI during the baseline and testing days. The analyses revealed significant main effects of Context test and Prepulse intensity, F(2,30)=5.55; p<.01, and F(2,30)=21.23; p<.001, respectively. The 2-way interaction was non-significant, F(4,60)<1. Pairwise comparisons (t-test for related samples, p<.05, two-tailed) revealed that the main effect of Prepulse Intensity was due to higher PPI for the 90 dB condition (mean = 49.32, SD = 8.62) as compared to the 80 dB and 100 dB conditions (mean = 35.21, SD = 11.52, and mean = 25.36, SD = 16.47, respectively).

The main effect of Context test on PPI is depicted in Figure 1. As can be seen in the figure, PPI was reduced when the test was conducted in presence of the context associated with amphetamine as compared to the baseline day. However, PPI was reinstated in spite of amphetamine administration when tested in presence of the context previously associated with haloperidol +

amphetamine injections. Pairwise comparisons between groups (t-test for related samples, one-tailed) revealed that PPI was reduced when comparing Baseline vs. Context amphetamine conditions, $t(15)=4.07$; $p<.001$, but remained intact when comparing Baseline vs. Context amphetamine + haloperidol, $t(15)=1.40$; $p>.09$. The difference between PPI measured in presence of Context amphetamine vs. Context haloperidol + amphetamine was close to the standard levels of significance, $t(15)=1.71$; $p>.058$. Finally, and in order to evaluate whether changes in PPI were related to variations in overall responding to the Pulse, an ANOVA was conducted on mean Startle to the pulse-alone trials with main factor Context test. The analysis revealed no significant differences, $F(2,30)=1.89$; $p>.17$.

Figure 1 about here

As predicted, the experimental results from the testing stage showed that the dopamine agonist reduced PPI when it was registered in the context previously associated with amphetamine as compared to baseline PPI [16,17]. Conversely, when PPI was registered in the presence of the context previously paired with haloperidol + amphetamine administration, it was expressed with the same intensity as in the drug-free baseline, in spite of the animals had been injected with amphetamine.

3. EXPERIMENT 2

The results of the first experiment revealed that the disruptive effect of amphetamine on PPI was counteracted when the sensory-motor gating effect

was registered in the presence of the context previously paired with haloperidol + amphetamine. We hypothesized that such a result was due to contextual conditioning of the dopaminergic activity, in such a way that context exposure induced a CR similar to the response activated by the dopamine antagonist. An alternative explanation of PPI modulation observed in Experiment 1 can be considered attending to a sensitization effect of the drug due to its repeated administration [35,36] Although the differences in PPI between the "Context amphetamine" and "Context amphetamine+haloperidol" conditions at testing argue against the sensitization hypothesis, testing was conducted in Experiment 2 free of drug to discard a possible sensitization effect on PPI.

Thus, the main purpose of Experiment 2 was to replicate the PPI modulation by context conditioning of dopaminergic activity observed in Experiment 1, but introducing some procedural changes intended to control the effect of possible uncontrolled variables. Specifically, and in order to avoid a possible effect of repeated measures, PPI was registered only at baseline and test trials in this experiment. A second change consisted of administering just haloperidol in the dopamine antagonist-conditioning condition, instead of haloperidol and amphetamine, as we did in Experiment 1. More specifically, in this experiment we included two groups receiving context-amphetamine (Group Amph) vs. context-haloperidol pairings (Group Hal). Thirdly, the number of context-drug pairings was changed to six, instead of three as in Experiment 1, to increase the magnitude of the expected CR. Finally, and as mentioned above, we conducted the final test stage drug-free to avoid drug sensitization effects, to evaluate contextual conditioning without any direct physiological effect on the dopaminergic activity, and to control for the possible role of state-

dependent learning in the effects found in Experiment 1. According to the results of Experiment 1, we predict that the context associated with amphetamine will generate a conditioned increase in dopamine activation that will reduce PPI intensity as compared to that observed during the drug-free baseline. Conversely, we expect an increase of PPI [37,33] when tested in the presence of the context associated with the dopamine antagonist.

3.1. Method

3.1.1. Subjects

16 male Wistar rats (n=8) experimentally naïve participated in this experiment. Mean weight at the start of the experiment was 426 g. (range 387-504). Food and water were available ad libitum throughout the experiment. Rats were individually housed in the colony with a regular light-dark cycle of 12:12 hours. Four days before the start of the experimental sessions, each of the animals was handled 5 minutes daily. All procedures were conducted in accordance with the guidelines established by Directive 86/609/CEE of the European Community Council, and the Spanish R.D. 223/1988.

3.1.2. Apparatus

The stimulus and apparatus were the same as described for Experiment 1.

3.1.3. Procedure

A summary of the experimental design is represented in the lower section of Table 1. The protocol to registered PPI was the same as described for Experiment 1. In order to obtain an index of PPI without the action of any drug, PPI was registered after an injection of saline solution the first two days of the experiment. On day 1, half of the animals from the Group Amph, and half

from the Group Hal were tested in Context A (mint/light). The second day, the other half of the rats on each group was tested for PPI in context B (almond/dark). Mean percent PPI on these sessions was considered as the baseline to compare with PPI at testing.

As described for baseline, contexts were counterbalanced. However, in order to simplify the procedure description we will not make reference to the different contexts when describing the experimental manipulations.

Context conditioning took place from day 3 to 26 and was organized in 4-day blocks. On each block, the animals in the Amph and Hal groups received amphetamine or haloperidol injections in the corresponding context on days 1 and 2. Immediately after drug administration, each animal was introduced in the conditioning context and remained undisturbed for 40 m. On days 3 and 4 of each 4-day block the rats were injected with a saline solution and introduced in the alternative context. This 4-day cycle was repeated for 6 times. PPI was measured after 3 and 6 context-drug pairings on days 3 and 4 of the cycle.

3.2. Results

A preliminary 3 x 2 x 2 mixed ANOVA (Prepulse intensity: 80 dB vs. 90 dB vs. 100 dB x Context: A vs. B x Group: Amph vs. Hal, the first two variables being within-subjects) was conducted on mean percent PPI registered during the first two days of the experiment (baseline). No main effects or interactions were significant (all ps>.25). The lack of significance reveals that there were neither differences induced by the changes in contextual cues nor differences in baseline for the Groups that subsequently received amphetamine and haloperidol.

In order to analyze the effect of context conditioning on PPI a 3 x 3 x 2 mixed ANOVA (Prepulse intensity: 80 dB vs. 90 dB vs. 100 dB x Test Day: Baseline vs. Test 1 vs. Test 2 x Group: Amph vs. Hal) was conducted on mean percent PPI. The analysis revealed significant main effects of Prepulse intensity and Group, $F(2,28)=10.21$; $p<.001$, and $F(1,14)=6.56$; $p<.05$, respectively. T-test for related samples ($p<.05$, two tailed) revealed that the main effect of Prepulse intensity was due to a lower mean PPI with the 100 dB prepulse (Mean = 40.35%, SD = 15.60) as compared to the 80 dB and 90 dB conditions (Mean = 52.76%, SD = 18.49, and Mean = 57.70%, SD = 17.30). The main effect of Group reflects lower PPI levels for those animals tested in the context paired with amphetamine as compared to those tested in the context associated with haloperidol (Mean = 42.22%, SD = 13.86, and Mean = 58.32%, SD = 11.14, respectively). The Test day x Prepulse intensity interaction was also significant, $F(4,56)=5.05$; $p<.01$. A detailed inspection of the interaction revealed that it was due to a lower percent PPI with the 100 dB prepulse intensity in the Test 1 and Test 2 trials. Finally, the Test day x Group interaction was also significant, $F(2,28)=4.26$; $p<.05$. No more main effects or interactions were significant.

The source of the Test day x Group interaction is depicted in Figure 2. As can be seen in the figure, mean percent PPI was reduced when registered in the context paired with amphetamine. Conversely, PPI increased when registered in presence of the context paired with haloperidol. These differences only appeared at second test trial. Specifically, t-test for independent samples (one-tailed) comparing mean percent PPI for Amph and Hal groups for Test 1 (conducted after three context-drug pairings) revealed the absence of

differences, t(14)=1.41; p=.09. For Test 2, after six context-drug pairings, PPI was more intense when tested in presence of the context previously paired with haloperidol than when it was tested in the amphetamine context, t(14)=3.59; p<.01. Additionally, we conducted within groups *a priori* comparisons based on our hypotheses (t-test for related samples, one-tailed). The analyses for the Group Amph revealed a significant reduction of PPI at test 2 as compared to test 1, t(7)=1.95; p<.05. There were no differences between PPI at baseline vs. test 1 or vs. test 2, t(7)<1, and t(7)=1.62; p=.074, respectively. As for the comparisons for the context-haloperidol Group, in spite of trend of increased PPI at test 1 and 2 as compared to baseline than can be observed in Figure 2, the differences between baseline, test 1 and test 2 were non-significant (all ps>.08). Finally, to assess whether changes in PPI were due to changes in overall responding to the pulse, a mixed ANOVA with main factors Test day x Group was conducted on mean startle to the Pulse-alone trials. The analysis only revealed a significant main effect of Test day, F(2,28)=3.73; p<.05, due to a reduction of startle intensity on the first and second test trials as compared to the baseline day. Neither the main effect of Group nor the 2-way interaction was significant (both ps>.33), revealing that the effect of the experimental treatment on PPI was not due to changes in overall response to the Pulse.

Figure 2 about here

The results showed reduced PPI in the Amph as compared to the Hal group when PPI was registered in the conditioned context. This effect was restricted to the second test trial that took place after six context-drug pairings.

Therefore, these results are consistent with those obtained in the first experiment, and add evidence to our proposal that dopamine activity can be modulated through a conditioning process.

4. Discussion

In summary, the present study demonstrated that a context repeatedly paired with a dopamine antagonist (Experiments 1) or with a dopamine agonist (Experiment 2) acquired the property to elicit a CR that is similar to that produced by the drug. In addition, we replicated the reduction of PPI by amphetamine administration [30,32], and the restoration of the amphetamine-mediated PPI reduction by haloperidol [32]. More importantly, PPI remained intact in spite of amphetamine administration when it was tested in the context that had been paired with haloperidol + amphetamine in Experiment 1. This result indicates that the dopaminergic activity elicited by the dopamine antagonist was associated with the presence of the contextual cues in such a way that the context at testing produced a conditioned reduction in dopamine that counteracted the effect of amphetamine, and normalized the PPI effect. The results of Experiment 2 are consistent with this perspective, since PPI was slightly increased when measured drug-free in the contextual cues that had been associated with a dopamine antagonist, although the differences were non-significant. Conversely, PPI was significantly disrupted when tested drug-free in the context that had been paired with the effects of the dopamine agonist.

There is previous evidence on conditioned dopamine modulation in experiments that evaluate locomotor activity in contexts associated with amphetamine [16,18,20,35], or cocaine injections [39]. Thus, for instance,

Beninger and Hahn [20] injected d-amphetamine (2.5 mg/Kg, i.p.) before introducing the rats in experimental chambers to register general activity. Next, the animals were injected with saline solution and returned to their home cages. In a control group, this treatment was reversed. When the animals were subsequently tested in the experimental chambers, those rats in the control group showed significantly less activity than those that had received the drug in the experimental chamber. A particularly relevant result in this domain was reported by Fontana Post & Pert [40], since they directly registered levels of dopamine after context conditioning. Specifically, they used the same procedure described above but injecting cocaine (40 mg/Kg) instead of amphetamine. At the behavioral level, they found an increase of rotational and stereotyped behaviors when the rats were tested in the context associated with the drug. At a physiological level, they found that exposing the animals to the conditioned context resulted in a dopamine increase in the Nucleus Accumbens extracellular space. Therefore, the described results, as well as our own experimental results, give strong support to the idea of conditioned-mediated increases and decreases of dopamine activity.

Some authors have proposed an alternative non-associative account based on the effect of dopamine agonists on perceived context novelty for the results obtained in the experiments on dopamine conditioning [16,38]. Specifically, it has been repeatedly demonstrated that a new environment elicits exploratory responses that increases motor activity [41]. On the other hand, amphetamine administration impedes context habituation [42], therefore it might be that repeated amphetamine administration could be maintaining the context as functionally novel on every exposure, and this effect would be the basis of

the proposed dopamine-mediated increase in motor activity. In an attempt to evaluate this possibility, Ahmed, Oberling, Di Scala and Sandner [38] introduced a phase of context familiarization before association with the contextual cues with the drug (1.25 mg/Kg amphetamine). The results revealed that, in spite of previous context familiarization, motor activity increased in the context-drug group as compared to the control group, thus discarding an explanation based exclusively on a context novelty interpretation.

The non-associative hypothesis could also be relevant to our results and, more specifically, to those groups that received amphetamine, since there is evidence that a novel stimulus presented at the time of PPI evaluation can reduce the intensity of the startle-modulation phenomenon [34,43]. As PPI in our experiments was registered at the test stage in the context that had been consistently presented after amphetamine injections, and amphetamine prevents context novelty habituation, it is possible that context novelty was the basis of reduced PPI. As discussed by Schmajuk, Larrauri, De la Casa and Levin [43], this reduction can be related to an increase in dopaminergic activity in the Nucleus Accumbens elicited by the novel environment [44].

However, there are several facts that rule out such a non-associative interpretation of our experimental results (particularly those from Group Amph in Experiment 2): first, if the novelty-based hypothesis is correct, we would expect reduced PPI in all groups from Experiment 1, because the test stage was conducted under the effects of amphetamine. However, we obtained exactly the opposite result when the test was conducted in the presence of the context associated with haloperidol + amphetamine. Secondly, the non-associative hypothesis cannot explain the intact PPI observed in Experiment 2 for the Amph

Group after three context-drug pairings (see Figure 2), because at this time the context novelty should be higher than after six pairings and, therefore, context novelty should be promoting reduced PPI.

In the introduction, we described the hypothesis proposed by Eikelboom and Stewart [5] that tries to make compatible the conflicting results observed in the analysis of drug conditioning (CR similar or opposite to the physiological effects of the drugs). According to this proposal, the results from our study indicate that both amphetamine and haloperidol produced their effects by an action on an efferent arm of the dopaminergic system, assuming that the changes observed in PPI were the result of increments or decrements in dopamine activity [23]. However, there is also previous research examining conditioning with amphetamine and haloperidol as an US that resulted in opponent or compensatory responses. Thus, for instance, Poulos, Wilkinson, and Cappell [45] conducted a series of experiments examining tolerance to amphetamine-induced anorexia that revealed the occurrence of a compensatory CR that counteracted the drug effect when it was administered in the same context associated with the drug administration. Similarly, tolerance to haloperidol-induced catalepsy in rats was observed only when the animals were exposed to the context previously associated with the drug [46]. Therefore, the different conditioned reactions to the same drug can be interpreted as an adaptive response produced to increase the survival of the organism, and the differences detected seem to be related to the specific responses selected on each experimental situation.

The present findings have implications for the therapeutic use of dopaminergic drugs, and more specifically, for adjustment of the appropriate

dose of antipsychotic drugs in psychiatric patients [47]. There are a large number of side effects of new and traditional neuroleptic drugs [48,49,50]. Considering the role of classical conditioning to generate a CR that mimics the effect of the drug, we can predict that maintaining constant all internal and external cues present at the time of treatment, an associative process will develop that activates anticipatory CRs similar to those induced for the drug. An appropriate schedule of conditioning, alternating context-drugs and context-placebo trials, could be programmed to reduce the drug dose but maintaining the therapeutic effectiveness of treatment.

Future studies are necessary to directly evaluate changes in dopamine levels in the presence of the CS associated with the drug. There is experimental evidence of a conditioned increase in extracellular dopamine after context-cocaine association [40], and higher concentrations of the dopamine metabolite homovanillic acid in mesolimbic and caudate regions after context-amphetamine and context-apomorphine pairings as compared to pseudo-conditioned control groups [51]. However, there are also conflicting results indicating no change in dopamine metabolite concentrations using cocaine as the CS [52]. Hopefully, understanding how the neuropsychological mechanisms are activated by both drug action and environmental associations will provide new strategies to approach the treatment of psychiatric syndromes characterized by malfunctioning of the dopaminergic system.

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Table 1. Summary of the design for Experiment 1 (upper section), and for Experiment 2 (lower section). A and B refer to two separate contexts (counterbalanced). Amph: Amphetamine. Hal: Haloperidol. PPI was registered during all sessions for Experiment 1. For Experiment 2 PPI was registered at Baseline, and after third and sixth conditioning trials. See text for additional details

Experiment 1

Baseline	Conditioning (3 cycles)	Test
A/B: Saline	A: Amph / B: Hal + Amph	A: Amph / B: Amph

Experiment 2

Group	Baseline	Conditioning (6 cycles)	Test (after 3rd and 6th conditioning cycle)
Amph	A/B: Saline	A: Amph / B: Sal	A: Sal / B: Sal
Hal	A/B: Saline	A: Hal / B:Sal	A: Sal / B: Sal

Figure captions.

Figure 1: Mean percent PPI collapsed across trials measured at the baseline session (with the animals injected with saline solution) and for those sessions conducted in presence of the context associated with amphetamine and the context associated with haloperidol + amphetamine (the animals received an amphetamine dose before the test trial). Error bars represent SEMs.

Figure 2: Mean percent PPI collapsed across trials as a function of context-drug conditioning (amphetamine vs drug) for Baseline, first test trial (after three context-drug pairings) and second test trial (after six context-drug trials). All tests were conducted without drugs. Error bars represent SEMs.

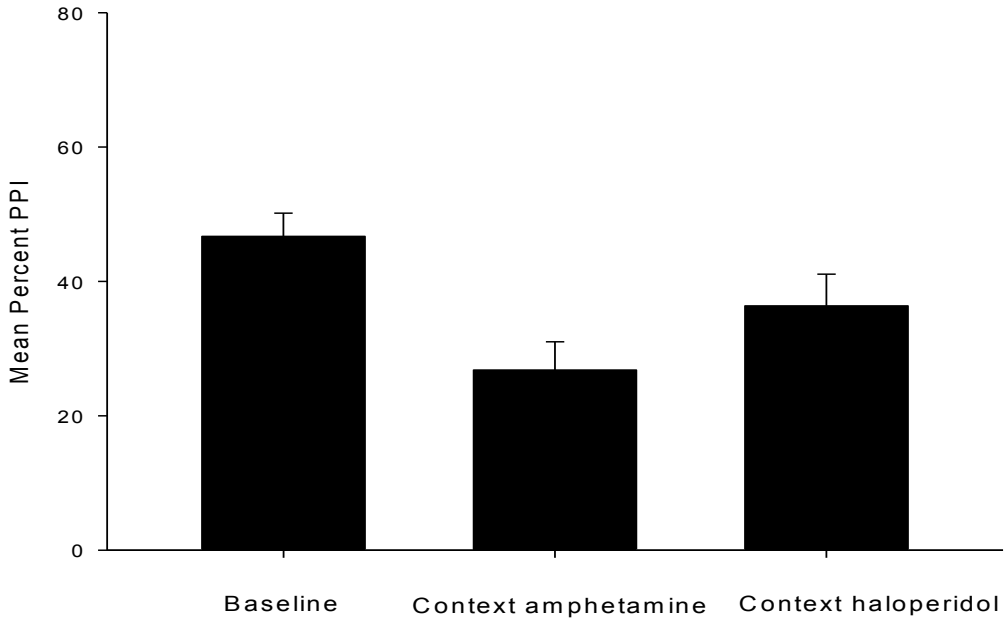
80
60
40
20
0
Mean Percent PPI
Baseline
Context amphetamine
Context haloperidol

Figure 1.

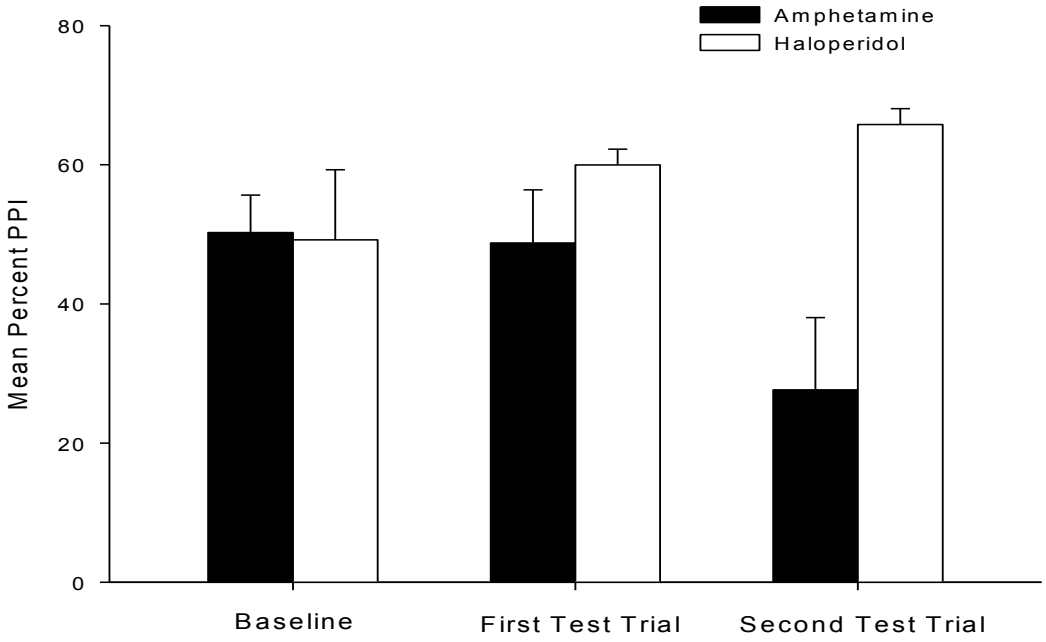
Amphetamine
Haloperidol
80
60
40
20
0
Mean Percent PPI
Baseline
First Test Trial
Second Test Trial

Figure 2.